JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Volatile Compounds Contributing to Naturally Occurring Fruity Fermented Flavor in Peanuts

JEFFREY L. GREENE,[†,§] TIMOTHY H. SANDERS,*[,#] AND MARY ANNE DRAKE[†]

Department of Food, Bioprocessing, and Nutrition Sciences, and MQHRU, ARS, USDA, 236 Schaub Hall, North Carolina State University, Raleigh, North Carolina 27695

Published research has indicated that ethyl 2-methylpropanoate, ethyl 2-methybutanaote, ethyl 3-methylbutanoate, hexanoic acid, butanoic acid, and 3-methylbutanoic acid are responsible for fruity fermented (FF) off-flavor; however, these compounds were identified in samples that were artificially created by curing immature peanuts at a constant high temperature. The objective of this study was to characterize the volatile compounds contributing to naturally occurring FF off-flavor. Volatile compounds of naturally occurring FF and no-FF samples were characterized using solvent-assisted flavor evaporation (SAFE), solid phase microextraction (SPME), gas chromatography−olfactometry (GC-O), and gas chromatography−mass spectrometry (GC-MS). Aroma extract dilution analysis (AEDA) identified 12 potent aroma active compounds, none of which were the previously identified esters, with no consistent differences among the aroma active compounds in no-FF and FF samples. Hexanoic acid alone was identified in the naturally occurring FF sample using the SAFE GC-MS methodology, whereas two of the three previously identified esters were identified in natural and artificially created samples. The same two esters were confirmed by SPME GC-MS in natural and artificially created samples. This study demonstrated the need for caution in the direct application of data from artificially created samples until those compounds are verified in natural samples. However, these results suggest that a laboratory method using SPME-GC techniques could be developed and correlated on an ester concentration versus FF intensity basis to provide an alternative to sensory analysis for detection of FF off-flavor in peanut lots.

KEYWORDS: Peanuts; fruity fermented; off-flavor; sensory analysis; GC-O; instrumental analysis

INTRODUCTION

The majority of peanuts (*Arachis hypogaea* L.) produced in the United States are used for human consumption, and the underlying basis for consumer purchase and consumption is the unique roasted peanut flavor. Off-flavors are of major concern to peanut manufacturers (*1*). A relatively common off-flavor in peanuts is described as fruity fermented (FF). Previous research has indicated that FF off-flavor is developed when immature peanuts are cured at temperatures in excess of 35 °C (*2*).

For the past 45−50 years, flavor chemists have studied the volatile compounds contributing to roasted peanut flavor. Early flavor research studies (*3–10*) generally utilized instrumental analysis alone as a basis for hypothesis of links between specific compounds and specific flavors; however, the understanding of flavor involves extensive sensory and volatile analysis. The types of compounds commonly reported were pyrazines, pyrroles, thiazoles, phenols, pyridines, ketones, aldehydes, terpenes, furans, esters, lactones, alcohols, and aromatic hydrocarbons.

More recent flavor chemistry research has incorporated descriptive sensory and instrumental analysis (*11, 12*). Phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine were reported as key volatile compounds responsible for stale/floral and ashy off-flavors in high-temperature microwave-blanched peanuts (*12*). Didzbalis et al. (*11*) identified ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, hexanoic acid, butanoic acid, and 3-methylbutanoic acid as the compounds responsible for FF off-flavor in immature peanuts artificially cured at a constant temperature of 40 °C.

To our knowledge, there are no reports of the compounds contributing to naturally occurring FF off-flavor in peanuts. The long-range basis for the study was to determine if an instrumental method could be developed to evaluate peanut lots for FF off-flavor intensity on the basis of the concentration of compounds reported to be responsible for the off-flavor. The specific objective of this study was to characterize the volatile compounds contributing to natural FF off-flavor using sensory analysis, instrumental analysis, and model systems evaluation.

* Corresponding author [telephone (919) 515-6312; fax (919) 513-8023; e-mail tim.sanders@ars.usda.gov].

[†] Department of Food, Bioprocessing, and Nutrition Sciences.

[§] Present address: Kraft Foods, 801 Waukegan Rd., Glenview, IL 60025.

[#] MQHRU, ARS, USDA.

10.1021/jf800450k CCC: $40.75
Published on Web 08/08/2008

MATERIALS AND METHODS

Sample Preparation. Natural FF Samples. Twenty, 1 ton, shelled, medium grade size, runner-type peanut lots (var. FlavorRunner 458) (FR 458) were each identified by a commercial peanut sheller as potentially containing naturally occurring FF off-flavor. The lots had been identified as having various intensities of FF off-flavor after sensory evaluation of one sample from each lot by a commercial laboratory. Each of the 20 lots was sampled at the sheller's location to obtain 117 kg, and these samples were then riffle-divided to obtain 20 subsamples of 680 g.

Natural FF off-flavor is defined here as the development of the off-flavor under naturally occurring cultural and handling practices to include maturity of the crop, inverted-windrow (field) drying, and approved curing practices with heated air. The 20 lots were produced in the same general area and during field drying were subjected to diurnal temperature variations with ca. 41 °C highs and 4 °C lows. Most of the lots were not subjected to heated air drying, although precise identification was not possible. A high percentage of peanut lots from the production location were eventually identified by extensive manufacturer testing to have FF off-flavor.

Peanut samples were roasted for 12 min at 176 °C using a laboratory-scale roaster (Aeroglide Corp., Raleigh, NC) to obtain a roast color of Hunter $L = 50 \pm 1$. Roasted peanuts were cooled using forced ambient air before seed coats were manually removed and peanuts (250 g) were processed into paste (*13*). Paste samples were stored at −4 °C and tempered to room temperature prior to sensory analysis. Each of the 400 samples (20 lots × 20 subsamples) was evaluated in duplicate by an experienced, eight-member descriptive sensory panel. All panel members had a minimum of 500 h of experience with peanut flavor and flavor variation. Some of the lots initially identified by the sheller as having FF off-flavor did not contain the off-flavor. A sample with relatively high FF off-flavor (intensity = 2.6) and a sample that did not have FF off-flavor (intensity = 0) were selected for further instrumental analysis.

Artificially Created FF Samples. Freshly harvested Georgia Green (GG) and FR 458 peanut varieties (45.3 kg) were obtained from USDA, ARS, National Peanut Research Laboratory (NPRL) research projects in Georgia and Texas, respectively. Samples were shipped in coolers overnight to Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC. Peanuts were sorted by mesocarp color into Pod Maturity Profile maturity classes after removal of the exocarp with a pressure washer (*14*). The mesocarp colors from mature to immature are black, brown, orange B, orange A, and yellow, respectively. Black and brown pods (BB) were used as the mature lot and orange A (only slightly orange) and yellow pods (OY) were used as the immature lot. One intermediate color, orange B (advanced orange), was not used in order to provide complete separation of mature and immature pods. BB and OY pods from each variety were each subjected to constant temperatures of both 27 and 40 °C using forced heated air in an oven until a seed moisture content of 8% was obtained following the published procedures of Didzbalis et al. (*11*). Peanut pods were shelled, and medium grade size peanuts (8.3 mm > width > 7.1 mm) were used for further evaluation. Exposure of these samples to constant temperatures of 27 and 40 °C constituted a totally artificial temperature regimen that cannot occur in a natural situation. The two natural samples selected from the 400 original samples were designated no-FF and FF, and the artificially created samples were designated GGOY27, GGOY40, GGBB27, GGBB40, FR458OY27, FR458OY40, FR458BB27, and FR458BB40 to indicate the variety, maturity class, and curing temperature. Samples cured at constant temperature were roasted and processed as previously described.

Sensory Evaluation of Peanuts. The trained sensory panel evaluated the samples using a lexicon (**Table 1**) developed for peanut flavor (*15*). The FF descriptor was first identified and added to the lexicon (*2*) in studies to examine the relationship of high temperature curing and maturity of peanuts. Each panel session consisted of the evaluation of seven samples, and descriptor intensities were scored using the Spectrum method on a 15-point universal intensity scale (*16*). Panelists expectorated the peanut samples after analysis, rinsed with water, and cleansed their palates with unsalted crackers as needed. Peanut samples were evaluated in duplicate.

Solvent Extraction Techniques. *Chemicals.* Ethyl ether (anhydrous, 99.8%), sodium chloride (99%), sodium sulfate (99%), 2-methyl-3-heptanone (internal standard for neutral/basic fraction), and 2-methylvaleric acid (internal standard for the acidic fraction) were obtained from Aldrich Chemical Co. (St. Louis, MO). Internal standard (3-methylvaleric acid) for the acidic fraction was obtained from Lancaster (Windham, NH). The reference standards for all aroma compounds were purchased from Sigma-Aldrich (St. Louis, MO). The sodium bicarbonate (99.7%) and hydrochloric acid (36.5%) were obtained from Fisher Scientific (Pittsburgh, PA).

Direct Solvent Extraction. One hundred grams of peanut paste was weighed and divided into four Teflon centrifuge bottles. An internal standard mixture (50 μL of 2-methyl-3-heptanone and 50 μL of 3-methylvaleric acid) was suspended in 5 mL of ethyl ether, and 15 μL was added to each centrifuge bottle. After the addition of the internal standard, 50 g of NaCl and 50 mL of ethyl ether were added to each bottle. Sample mixtures were shaken for 30 min on a Roto mix (Barnstead/Thermolyne type 50800; Dubuque, IA). The bottles were centrifuged in a Sorvall RC-5B refrigerated (3.0 °C) superspeed centrifuge (DuPont Instruments) for 15 min at 1207*g*. The solvent phase was collected, and the procedure was repeated three times with the addition of 50 mL of ethyl ether to each bottle each time. The combined solvent phases were combined and stored at −20 °C until further analysis.

Solvent-Assisted Flavor Evaporation (SAFE). Volatile compounds were separated from the solvent extracts using SAFE (*17*). Samples were vacuum distilled on the SAFE apparatus as previously described (*12*). After the sample was completely introduced into the SAFE system, the distillation was carried out for 2 h at 10^{-4} torr. Volatile compounds were collected in a trap, which was submerged in liquid nitrogen. The volatile distillate was removed and reduced to a final concentration of 20 mL with a gentle stream of nitrogen gas.

Phase Separation (Neutral/Basic and Acidic Fractions). The concentrated volatile distillates were washed and vortexed twice with 3 mL of 0.5 M sodium bicarbonate and three times with 2 mL of saturated sodium chloride. After each wash, the water phase (bottom layer) was transferred to a screw-cap tube. The remaining ether phase was designated the neutral/basic fraction. The pH of the collected water phase was lowered to pH 2.0−2.5 with 18% HCl and extracted with ethyl ether (three times). After each ether addition, the ether phase (top layer) was removed and collected to constitute the acidic fraction. Each fraction was filtered through powdered sodium sulfate (three times) to remove any water in the extracts before each fraction was reduced to 0.5 mL under a stream of nitrogen gas.

Gas Chromatography−Olfactometry (GC-O) of SAFE Fractions. Peanut extracts (neutral/basic and acidic fractions) were analyzed on an HP5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector (FID) and a sniffer port. Two microliters of each fraction was evaluated on a nonpolar capillary column (DB-5 ms, 30 m length × 0.25 mm i.d. × 0.25 μm, d_f; J&W Scientific, Folsom, CA) and a polar capillary column (DB-Wax, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness, d_f; J&W Scientific). The column effluent was split (1:1) between the FID and sniff port. Nasal dehydration was reduced by combining 30 mL/min of humidified air with the GC effluent (*18*). The GC oven temperature was programmed from 40−200 °C at a rate of 8 °C/min with initial and final hold times of 5 and 20 min, respectively. Six experienced panelists conducted post peak aroma intensity analysis of the neutral/basic and acidic fractions twice on the DB-5 ms and DB-Wax columns. As compounds eluted from the column, the panelist described the detected odor and the intensity of the odor using a 10-point numerical intensity scale (*18*). Aroma extract dilution analysis (AEDA) was subsequently applied to determine the number of 1:3 dilutions required until the aroma of a compound was not detected in order to identify key odorants (*19*). The flavor dilution (FD) factor was defined as the highest dilution at which an odor was detected by GC-O. Higher FD values suggest that the particular compound contributes to the flavor or off-flavor of interest.

Gas Chromatography−Mass Spectrometry (GC-MS) of SAFE Fractions. Peanut extract fractions were separated on an Agilent Technologies 6890N GC/Agilent Technologies 5973 mass selective detector

Table 1. Lexicon of Peanut Flavor Descriptors[a]

term	definition
Aromatic	
roasted peanutty	aromatic associated with medium-roast peanuts (about 3−4 on USDA color chips) and having fragrant character such as methylpyrazine
raw bean/peanutty	aromatic associated with light-roast peanuts (about 1−2 on USDA color chips) and having legume-like character (specify beans or pea if possible)
dark-roasted peanut	aromatic associated with dark-roasted peanuts (4+ on USDA color chips) and having very browned or toasted character
sweet aromatic	aromatics associated with sweet material such as caramel, vanilla, molasses, fruit (specify type)
woody/hulls/skins	aromatics associated with base peanut character (absence of fragrant top notes) and related to dry wood, peanut hulls, and skins
cardboard	aromatic associated with somewhat oxidized fats and oils and reminiscent of cardboard
painty	aromatic associated with linseed oil, oil-based paint
fruity fermented	aromatic associated with overripe fruit
rotten garbage/soured	aromatic associated with old garbage
burnt	aromatic associated with very dark roast, burnt starches, and carbohydrates (burnt toast or espresso coffee)
green	aromatic associated with uncooked vegetables/grass twigs, *cis*-3-hexanal
earthy	aromatic associated with wet dirt and mulch
grainy	aromatic associated with raw grain (bran, cod liver oil, old fish)
fishy	aromatic associated with trimethylamine, cod liver oil, or old fish
chemical/plastic	aromatic associated with plastic and burnt plastics
skunky/mercaptan	aromatic associated with sulfur compounds, such as mercaptan, which exhibit skunk-like character
Tastes	
sweet	taste on the tongue associated with sugars
sour	taste on the tongue associated with acids
salty	taste on the tongue associated with sodium ions
bitter	taste on the tongue associated with bitter agents such as caffeine or quinine
Feeling Factors	
astringent	chemical feeling factor on the tongue, described as puckering/dry and associated with tannins and aluminum
metallic	chemical feeling factor on the tongue described as flat, metallic, and associated with iron and copper

[a] Adapted from Johnsen et al. (*15*) and Sanders et al. (*13*).

equipped with a fused nonpolar capillary column (DB-5 ms, 30 m length × 0.25 mm i.d. × 0.25 μm, d_f; J&W Scientific). Helium was used as the carrier gas at a constant flow of 1 mL/min. Oven temperature of the GC-MS was programmed from 40− to 200 °C at a rate of 2 °C/min with initial and final hold times of 5 and 30 min, respectively. The conditions of the mass selective detector were as follows: capillary direct interface temperature, 250 °C; ionization energy, 70 eV; mass range, 35−300 amu; EM voltage (Atune + 200 V); scan rate, 5 scans/s. One microliter of each extract was injected in duplicate using splitless mode.

Headspace Extraction Techniques. *Solid Phase Microextraction (SPME) GC-MS.* Highly volatile compounds from the natural and artificially created FF samples were extracted by SPME. Ten grams of peanut paste was measured into 20 mL clear screw-cap vials, and 5 μL of the internal standard was added (50 μL of 2-methyl-3-heptanone and 50 μL of 2-methylvaleric acid in 5 mL of ether). Headspace volatiles were analyzed in duplicate using a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) Stableflex fiber on a CTC Analytics CombiPAL system autosampler (Palo Alto, CA). Prior to injections, the samples were agitated at 40 °C (250 rpm) for 30 min to release the volatiles into the headspace. After agitation, the fiber was exposed in the headspace for 30 min. After absorption of the volatiles by the fiber, volatiles were thermally desorbed from the fiber for 5 min and injected onto the GC-MS. An Agilent Technologies 6890N GC/Agilent Technologies 5973 mass selective detector equipped with a fused nonpolar capillary column (DB-5 ms, 30 m length × 0.25 mm i.d. × 0.25 μm, d_f; J&W Scientific) was used for separation. One microliter of each sample was injected in the splitless mode in duplicate, and the oven temperature was programmed from 40 to 250 °C at a rate of 8 °C/min with initial and final hold times of 5 min. Helium was used as the carrier gas with a constant flow of 1 mL/min. The mass selective detector conditions were the same as previously described. In single-ion monitoring (SIM) mode the selective ions for the esters were *m/z* 102, ethyl 2-methylbutanoate; *m/z* 88, ethyl 3-methylbutanoate; and *m/z* 116, ethyl 2-methylpropanoate.

Identification of Odorants. Tentative identifications were obtained by comparing mass spectra of authentic standard compounds to unknown compounds using the mass spectral database of the National Institute of Standards and Technology (*20*). Additionally, the retention indices (RI) and odor of unknown compounds were compared to the Flavornet database (http://www.flavornet.com) and those of authentic standard compounds analyzed under the same conditions. Positive identifications were accomplished by comparing the mass spectra, RI, and odor of unknowns with those of authentic standards under identical conditions. Retention indices (RI) were calculated using an *n*-alkane series (*21*).

Quantification of Volatile Compounds. Selected volatiles were quantified by calculating the relative abundance of the selected compounds. The peak area and concentration of the internal standard with the peak area of the compound were used: (relative abundance = peak area IS/peak area compound × [IS]). Standard curves were generated for 2-methylbutanal, trimethylpyrazine, and hexanoic acid. These compounds were selected because they represented the different compound groups found in the peanut samples. The selected compounds were quantified by analysis of standards in deodorized water using SPME and GC-MS, and a five-point standard curve was generated for each compound. Results indicated 2-methylbutanal and trimethylpyrazine had a linear fit of $R^2 > 0.97$, whereas hexanoic acid had $R^2 > 0.99$.

Statistical Analysis. Analysis of variance (ANOVA) using the general linear model (GLM) was used to determine differences among the sensory data. Fisher's LSD was the posthoc test used to determine differences among the sample means (SAS version 9.1, Cary, NC).

Table 2. Sensory Analysis of Natural and Artificially Created Fruity Fermented (FF) Off-Flavor in Georgia Green and Flavor Runner 458 Peanut Varieties[a]

sample[b]	roast peanutty	sweet aromatic	dark roast	raw beany	woody/hulls/skins	sweet taste	bitter	astringency	fruity fermented	cardboardy
no FF	4.2ab	3.6ab	3.6a	1.7e	3.0bc	3.7a	2.2c	1.0d	0.0d	0.0bc
natural FF	3.6bc	2.9cd	3.4ab	2.3d	3.2ab	2.7bc	2.9b	1.10cd	2.6b	1.1b
GGBB27	2.0e	1.7fg	2.50c	2.7a	3.3a	1.5d	3.6a	1.3b	0.0d	2.0a
GGBB40	2.2e	1.5g	2.7c	2.7a	3.3a	1.6d	3.8a	1.4a	0.0d	2.3a
GGOY27	3.6d	2.0ef	2.8bc	2.6abc	3.1ab	1.5d	3.5a	1.2bc	0.0d	1.1b
GGOY40	2.9d	2.3e	2.7c	2.6ab	3.2ab	1.9cd	3.7a	1.1cd	1.1cd	0.0cd
FR458BB27	4.6a	3.70a	3.1abc	2.2bcd	3.1ab	3.7a	2.4bc	1.0d	0.0d	0.0d
FR458BB40	4.3a	3.3bc	3.0abc	2.2cd	3.1ab	3.3ab	2.5bc	1.0d	2.2bc	0.0cd
FR458OY27	4.0ab	3.0cd	2.90bc	2.4abcd	3.2ab	3.0ab	2.9b	1.0d	2.3ab	0.0d
FR458OY40	3.2cd	2.8d	2.8bc	2.1de	2.8c	2.5bc	2.9b	1.1cd	3.4a	0.0cd

[a] Means in the same column with different letters are significantly different ($P < 0.05$). Flavor intensities were scored using the Spectrum method (*16*) on a 15-point universal intensity scale, where 0 = absence of the attribute and 15 = very high intensity of the attribute. [b] GG, Georgia Green; FR458, Flavor Runner 458; BB, black and brown (mature peanuts); OY, orange and yellow (immature peanuts); 27, curing temperature (°C); 40, curing temperature (°C).

RESULTS AND DISCUSSION

Sensory Evaluation. Flavor differences were evident among the natural and created FF samples. The sensory panel characterized the natural FF sample and some of the created samples as having a sweet, overripe fruit flavor characteristic of FF off-flavor as defined in the peanut lexicon (*2, 15*). In contrast, the panel often described the 40 °C immature FR 458 samples as having a rotten garbage/soured off-flavor. We hypothesize that the rotten garbage/soured flavor was developed when high-moisture, immature peanuts were exposed to a constant high curing temperature. All currently used curing methods include diurnal variation, which might include high temperature for a short period of time. However, no natural (field) or other currently used curing practice can produce a constant temperature. Although 27 °C is not a high temperature for current peanut curing methods, the low level of FF produced in immature FR 458 peanuts at 27 °C is also possibly due to the application of constant rather than diurnal temperature.

In a previous study (*11*) the separate terms fruity and fermented were used in the sensory evaluation of FR 458 peanuts that had been subjected to a constant 40 °C to create FF samples. The published lexicon term is fruity fermented (*2*). The use of two separate terms was not explained, and verbal definitions of the individual descriptors, fruity and fermented, were not provided (*11*). However, the authors did indicate that the 40 °C cured immature samples had high intensities of fruity, fermented, and sour notes. They further indicated that the addition of organic acids in a model system resulted in increased fermented notes and that the addition of esters and organic acids increased the sensory perception of fruity and fermented notes. Although not confirmed, it is possible that our term rotten garbage/soured flavor was similar to the term fermented in the previous paper (*11*). For the purpose of this study, the panel used only the term FF even though verbal descriptions for some samples were rotten garbage/soured.

FR458OY40 had the highest FF intensity (3.4) and was significantly ($P < 0.05$) different from the natural and GG samples (**Table 2**). The GGOY40 sample was the only GG sample identified as having FF off-flavor and the intensity was 1.1, which is slightly above the threshold of detection. We hypothesize that the difference in development of FF in the GG and FR 458 samples was related to naturally occurring differences in sugar concentrations between them. The FR458 samples were grown in an area with lower night temperatures, which consistently results in higher sugar concentrations (*25*). The natural FF sample had an intensity of 2.6 and was not significantly different ($P < 0.05$) from the FR458OY27 sample. This indicates that even at low temperatures (27 °C) the immature FR458 peanuts are more susceptible to formation of FF off-flavor; however, that temperature was applied constantly. A previous study investigated the effect of curing temperature on descriptive flavor of peanuts from different pod color maturity classes (*2*). Results of that study also indicated slightly increased intensities of FF, sour, and bitter in immature peanuts cured at ambient diurnal temperature (maximum 28 °C and minimum 22 °C). Additionally, that study indicated that increasing heated air curing temperatures resulted in higher intensities of FF, sour, and bitter in immature peanuts compared to mature peanuts. The results of the current study are consistent with the results reported previously (*2*).

The FR458BB27 sample had the highest roasted peanutty intensity (4.6) and was not significantly different from the no-FF sample, FR458BB40, and FR458OY27. GGBB27 and GGBB40 had the lowest roasted peanutty intensities of 2.0 and 2.2, respectively. The GG samples were the only samples with measurable cardboardy flavor, which indicated the beginning of lipid degradation. Several studies have indicated a decrease in roasted peanutty when descriptors such as cardboardy and painty begin to increase (*22, 23*). In the present study, the natural and artificially created FR 458 samples were significantly ($P < 0.05$) higher in sweet aromatic and sweet taste compared to the GG samples. Among the GG samples, GGOY40 had the highest intensity of sweet aromatic (SA) (intensity = 2.3) and sweet (intensity = 1.9). For the FR 458 samples, FR458OY40 had the lowest SA intensity (2.8). This low SA intensity may be related to the fact that FR458OY40 had the highest FF intensity (sample most commonly described as rotten garbage/soured flavor). In the natural samples, the no-FF sample had significantly ($P < 0.05$) higher SA and sweet taste intensities.

Aroma-Active Volatile Compounds Determined by SAFE. Post peak intensity analysis is useful in determining aroma-active compounds that are present in the sensory threshold range and the compounds that are aroma-active in a sample. However, it is difficult to determine which compound(s) potentially relate to flavor because the presence of a compound is not always indicative of contribution to a particular flavor. One hundred and sixty-one aroma-active compounds were detected from the neutral/basic peanut fractions, which is consistent with previous studies (*12*). The volatile profiles (GC) and odor intensities (GC-O) of the no-FF and FF sample were similar (data not shown). AEDA is a dilution screening technique that helps to identify important aroma-active compounds. Compounds with high FD factors are generally important in a particular flavor and are normally examined further (*21*). Among the 12 highly aroma-active compounds identified in the neutral/basic fractions of the no-FF and the FF samples, there were 4 pyrazines, 4 aldehydes, 2 ketones, 1 pyrroline, and 1 phenol (**Table 3**). Seven compounds were positively identified, and five were tentatively

Table 3. Neutral/Basic Aroma-Active Compounds in Natural Fruity Fermented (FF) Peanuts with High Flavor Dilution Factors As Determined by Aroma Extract Dilution Analysis

no.	compound	RI DB-MS[a]	RI DB-Wax[a]	GC-O odor	mean ($\log_3$ FD)[b] no FF (0 FF intensity)	mean ($\log_3$ FD)[b] FF (2.6 FF intensity)	identification method[c]
1	2,3-butadione	610	979	buttery/butterscotch	3.0 (4)	2.6 (4)	odor, RI[d]
2	3-methylbutanal	642	910	malty/chocolate	3.0 (5)	2.5 (<1)	odor, RI, MS
3	hexanal	803	1023	green/grassy	3.1 (5)	2.9 (4)	odor, RI, MS
4	methional	921	1472	potato	4.3 (6)	3.9 (6)	odor, RI[d]
5	2-acetyl-1-pyrroline[e]	936	1322	popcorn	4.0 (6)	4.0 (7)	odor, RI[d]
6	1-octen-3-one[e]	987	1267	metallic/mushroom	3.3 (5)	3.5 (4)	odor, RI[d]
7	2-ethyl-6-methylpyrazine	1007	1499	sweet	3.1 (2)	3.8 (3)	odor, RI, MS
8	trimethylpyrazine	1015	1452	earthy/soil/dirt	4.0 (6)	4.0 (5)	odor, RI, MS
9	phenylacetaldehyde	1063	1680	rosy/floral	5.4 (7)	4.6 (7)	odor, RI, MS
10	2-ethyl-3,5-dimethylpyrazine	1092	1479	earthy/soil/dirt	3.5 (9)	3.7 (9)	odor, RI, MS
11	2,3-diethyl-5-methylpyrazine	1167	1538	earthy/soil/dirt	2.0 (9)	3.5 (10)	odor, RI, MS
12	2-methoxy-4-vinylphenol	1396	ND	licorice/sweet	3.6 (5)	3.3 (6)	odor, RI[d]

[a] Retention indices on DB-5MS (nonpolar) and DB-Wax (polar) columns. [b] Mean intensities of experienced panelists and flavor dilution factors. [c] Compound identified by RI, odor character, and MS in comparison with authentic standards under identical conditions. [d] Compound tentatively identified using RI and odor character. [e] All compounds except tentativley identified compounds **5** and **6** have been previously reported in ref *4–12, 25, and 26*.

Table 4. Acidic Aroma-Active Compounds in Natural Fruity Fermented (FF) Peanuts with High Flavor Dilution Factors As Determined by Aroma Extract Dilution Analysis

no.[a]	compound	RI DB-5[b]	RI DB-Wax[b]	GC-O odor	mean ($\log_3$ FD)[c] no FF (0 intensity)	mean ($\log_3$ FD)[c] FF (2.6 intensity)	identification method[d]
1	2-ethyl-3,5-dimethylpyrazine	1479	1092	earthy/soil/dirt	2.50 (3)	3.50 (3)	odor, RI, MS
2	acetic acid	628	1454	vinegar/acetic acid	2.88 (3)	2.75 (3)	odor, RI, MS
3	methional	903	1472	potato	2.63 (4)	3.38 (4)	odor, RI[e]
4	butanoic acid	881	1635	sweaty/musty/cheesy	1.50 (2)	2.13 (2)	odor, RI, MS

[a] Compounds **1**−**4** have been previously reported from peanuts. [b] Retention indices on DB-5MS (nonpolar) and DB-Wax (polar) columns [c] Mean intensities of experienced panelists and flavor dilution factors [d] Compound identified by RI, odor character and MS in comparison to the authentic standard under identical conditions [e] Compound tentatively identified using RI and odor character.

identified. All of the positively identified compounds and three of the tentatively identified compounds have been reported previously (*4–12, 24, 25*), and only the tentatively identified compounds, 2-acetyl-1-pyrolline and 1-octen-3-one, were not found in previous papers. The mean intensities of the compounds were higher in the no-FF sample except for 1-octen-3-one (**6**), 2-ethyl-6-methylpyrazine (**7**), 2-ethyl-3,5-dimethylpyrazine (**10**) and 2,3-diethyl-5-methylpyrazine (**11**). The intensities of 2-acetyl-1-pyrroline (**5**) and trimethylpyrazine (**8**) were equal in both samples. However, a comparison of the FD factors of compounds from the no-FF and FF samples did not suggest meaningful differences. 2,3-Butadione (**1**), methional (**4**), phenylacetaldehyde (**9**), and 2-ethyl-3,5-dimethylpyrazine (**10**) had the same FD factors in both the no-FF and FF samples. 3-Methylbutanal (**2**), hexanal (**3**), 1-octen-3-one (**6**), trimethylpyrazine (**8**), and 2-methoxy-4-vinylphenol (**12**) had higher FD factors in the no-FF sample, whereas 2-acetyl-1-pyrroline (**5**), 2-ethyl-6-methylpyrazine (**7**), and 2,3-diethyl-5-methylpyrazine (**11**) had higher FD factors in the FF sample. In the acidic fractions (**Table 4**) 2-ethyl-3,5-dimethylpyrazine (**1**), acetic acid (**2**), methional (**3**), and butanoic acid (**4**) had FD factors of ≥ 2.

The greatest difference between the no-FF sample and the FF sample was that the FD factors for 3-methylbutanal were 5 and <1, respectively. In previous research, the presence of 3-methylbutanal has been identified as a contributor to nutty flavor in Cheddar cheese (*26*). AEDA was used to investigate the volatile compounds contributing to the flavor of microwave-blanched peanuts (*12*). Although 3-methylbutanal was not identified as a contributor to flavor in microwave-blanched peanuts, the compound was found to have a relatively high FD factor. The lower intensity and FD factor of 3-methylbutanal in the FF sample may be related to the lower roasted peanutty (nutty flavor) intensity commonly found in peanuts with FF off-flavor.

Table 5. Relative Abundance of FF Esters Identified in Natural and Artificially Created Fruity Fermented Samples Determined by SPME-GC-MS

	relative abundance (μg/kg)			
compound	no FF	FF	GGOY40	FR458OY40
ethyl 2-methylpropanoate	ND[a]	ND	ND	ND
ethyl 2-methylbutanoate	ND	0.11 ± 0.02	1.33 ± 0.19	2.38 ± 0.21
ethyl 3-methylbutanoate	0.10 ± 0.02	0.37 ± 0.05	0.59 ± 0.06	5.26 ± 0.54

[a] ND, not detected.

Ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, hexanoic acid, butanoic acid, and 3-methylbutanoic acid were reported to be responsible for FF off-flavor in immature peanuts cured at a constant temperature of 40 °C (*11*). Aromatic compounds indicative of these esters and acids were not detected by GC-O of the SAFE extracts of the natural FF sample. In common flavor chemistry methods, GC-MS is used on only the compounds that are identified by aroma on GC-O. On the basis of the concentrations reported by Didzbalis et al. (*11*), identification of the compounds in natural samples by GC-O was anticipated. The fact that the aromas were not detected initially by GC-O suggested that those compounds may not have been present and were related only to artificially created FF samples. Coelution of the esters identified by Didzbalis et al. (*11*) with volatile flavor active and nonflavor active interfering compounds may also be responsible for their absence from sensory GC-O analysis. Despite this interference, SIM mode analysis revealed the two most prominent esters, ethyl 2-methylpropanoate and ethyl 2-methylbutanoate, in the SAFE extracts of the FF sample (**Table 5**).

Volatile Compounds Detected by SPME. SPME was used as an alternative method to rapidly screen natural and artificial FF samples. Natural FF and no-FF peanuts were evaluated by

SAFE, and both natural and artificially created FF peanuts were examined by SPME to further characterize headspace and highly volatile compounds to compare the possible sources of this off-flavor between natural and artificially created samples. Twenty-four volatile compounds were identified by SPME, and for the natural samples, 21 of the 24 compounds were slightly higher in the FF sample compared to the no-FF sample (data not presented). In previous research, ethanol has been used as an indicator of FF in peanuts that have been improperly cured. Ethanol, acetaldehye, ethyl acetate, formaldehyde, acetone, isobutyraldehyde, isovaleraldehyde, 2-methylvaleraldehyde, methyl butyl ketone, and hexanal were identified in high-temperature-cured peanuts, and ethanol has been used as marker compound for FF (*3*). In this study ethanol was not present in the no-FF sample or any of the BB (mature) samples (data not presented). Ethanol was not present in these particular samples because FF is related to both immaturity and high-temperature curing (*2*). However, ethanol was detected in increasing concentrations in the FF sample, the GGOY40, and the FR458OY40 artificially created samples, respectively.

The SPME results among the natural and artificially created samples were different for the three previously identified esters (*11*) (**Table 5**). FR458OY40 had the highest concentrations of ethyl 2-methylbutanoate and ethyl 3-methylbutanoate at 2.38 $\pm$ 0.21 and 5.26 $\pm$ 0.54 ppb, respectively. The GGOY40 sample had lower concentrations of ethyl 2-methylbutanoate and ethyl 3-methylbutanoate, whereas ethyl 2-methylpropanoate was not detected in the natural and artificially created samples. In the natural FF sample, ethyl 3-methylbutanoate was detected at lower levels (0.37 $\pm$ 0.05 ppb) than the artificially created FF samples, and the no-FF sample had the lowest concentration of ethyl 3-methylbutanaote at 0.10 $\pm$ 0.02 ppb ($P < 0.05$). Esters are described as having fruity/apple-like aromas, and the higher concentrations of these compounds in FR458 samples may be due to greater anaerobic respiration leading to higher ethanol content. Esters are formed by a reaction between an alcohol and a carboxylic acid. Butanoic acid and hexanoic acid, short-chain organic acids present in peanuts may react with alcohols to produce various types of esters. More ethanol is generated in peanuts cured at 40 °C during anaerobic respiration; therefore, the production of esters should be more efficient. The relationship between high sugar content in FR458 samples (*23*) and the formation of FF off-flavor and the precise phenomenon/mechanism of FF off-flavor development has not yet been determined. However, we hypothesize that higher sugar concentrations may relate to more ethanol production under anaerobic conditions, which would provide more reactants for the alcohol and organic acids reaction to produce esters responsible for FF.

The differences in volatile profiles of natural and artificially created FF samples is probably caused by the temperature difference (variable vs continuous high temperature), the differences in peanut maturity (generally mature vs immature), and the initial moisture content of the peanuts. Typically, peanuts are harvested when a high percentage of mature peanuts are on the plants, and they are placed in windrows for 1−3 days, wherein moisture content decreases to 20−25%. In some locations windrow drying is used exclusively to dry peanuts to about 10% moisture content. Windrow-dried (20−25% moisture content) peanuts are normally dried with <35 °C heated air in drying wagons to 10% moisture content. During this process, temperatures of >35 °C for a significant amount of time, especially early in the curing process, generally result in natural FF development, especially in the immature peanuts in the harvested lot (*2*). The artificially created samples were cured at constant temperatures over a period of time, and these conditions cannot happen in nature. These cumulative differences in natural versus artificial curing techniques logically affected the production and final concentration of the esters responsible for FF off-flavor.

Previous research has indicated that during high-temperature curing the rate of oxygen diffusion into the peanut seed is not sufficient to support aerobic respiration, which results in anaerobic respiration and the production of ethanol (*27*). As described earlier, esters are formed when alcohols and carboxylic acids react. The exaggerated curing conditions used to produce the artificially created samples would result in much more ethanol production in peanuts than natural conditions, resulting in FF peanuts. Thus, less ethanol would be present in the naturally occurring FF peanuts to react with the short-chain organic acids, which could result in very low, possibly non-detectable, concentrations of the esters.

In comparison to the esters previously identified (*11*), ethyl 3-methylbutanaote was the only compound detected in the natural no-FF sample, whereas ethyl 2-methylbutanoate and ethyl 3-methylbutanoate were both detected in the natural FF sample and the artificially created samples. The concentrations of esters in 40 °C cured immature peanuts found by SPME (**Table 5**) in this study were higher than the concentrations previously reported (*11*) using SAFE.

Model System Flavor Evaluation. Model systems are an integral part of confirming that specific compounds are responsible for a certain off-flavor. Descriptive sensory analysis indicated flavor differences between natural FF and artificially created FF samples. The artificially created FR458OY40 samples were often described by an experienced panel as having a harsh rotten garbage/soured off-flavor. Natural FF off-flavor was perceived as sweeter and associated with overripe fruit as defined in the peanut lexicon.

Ethyl 2-methylpropanoate (0.09 ppb), ethyl 2-methylbutanoate (0.13 ppb), ethyl 3-methylbutanoate (0.11 ppb), hexanoic acid (0.17 ppb), butanoic acid (0.55 ppb), and 3-methylbutanoic acid (3.04 ppb) were used in peanut paste to create model systems (*11*). When these previously reported concentrations (*11*) were used, most panelists described the typical rotten garbage/soured off-flavor found in FR458OY40 samples. Although the difference in flavor profiles was consistently described by the panel, they suggested that there may be a relationship or intensity continuum of the off-flavors found in natural and artificially created FF when different concentrations of the reported compounds were used in model systems (data not presented). These results suggest that at least two of the esters previously identified in artificially created FF samples are responsible for naturally occurring FF off-flavor, although the compounds were detected at only low levels in naturally occurring FF off-flavor samples. Model systems employed in the earlier work (*11*) did not include ethyl 2-methylpropanoate, and that compound was not identified in this study. Because the two esters were detected at only very low levels in the natural FF samples, these results further suggest the need for verification of odorants in natural samples when laboratory-created samples are used as the basis for identification of off-flavor odorants. Sensory results indicated that natural and artificially created fruity fermented samples had a range of FF which appeared to culminate at soured/rotten garbage off-flavor. On the basis of concentrations of esters used in model systems, the potential for a continuum of the off-flavor from fruity fermented to rotten garbage/soured is high, a fact

indicated by comments of panelists examining model systems containing those compounds.

Sensory panel methods to detect FF in commercial peanut lots are expensive and time-consuming. The evaluation of numerous samples from a lot would improve the accuracy of identification of FF peanut lots (*28*). Development of an alternative, dependable method for FF evaluation would be a significant advancement in quality evaluation of peanuts. SPME-GC methods in this study did identify two of the esters reported to be responsible for FF, and the results suggest that a laboratory method using this technique could be developed and correlated on an ester concentration versus FF intensity basis to provide an alternative to sensory analysis.

LITERATURE CITED

(1) Sanders, T. H.; Vercellotti, J. R.; Bett, K. L.; Greene, R. L. The role of maturation in quality of stackpole-cured peanuts. *Peanut Sci.* **1997**, *24*, 25–31.

(2) Sanders, T. H.; Vercellotti, J. R.; Blankenship, P. D.; Crippen, K. L.; Civille, G. V. Interaction of maturity and curing temperature on descriptive flavor of peanuts. *J. Food Sci.* **1989**, *54*, 1066–1069.

(3) Pattee, H. E.; Beasley, E. O.; Singleton, J. A. Isolation and identification of volatile components from high-temperature-cured off-flavor peanuts. *J. Food Sci.* **1965**, *30*, 388–392.

(4) Walradt, J. P.; Pittet, A. O.; Kinlin, T. E.; Maralidhara, R.; Sanderson, A. Volatile components of roasted peanuts. *J. Agric. Food Chem.* **1971**, *19*, 972–979.

(5) Johnson, B. R.; Waller, G. R.; Burlingame, A. L. Volatile components of roasted peanuts: basic fraction. *J. Agric. Food Chem.* **1971**, *19*, 1020–1024.

(6) Johnson, B. R.; Waller, G. R.; Foltz, R. L. Volatile components of roasted peanuts: neutral fraction. *J. Agric. Food Chem.* **1971**, *19*, 1025–1027.

(7) Singleton, J. A.; Pattee, H. E.; Johns, E. B. Influence of curing temperature on the volatile components of peanuts. *J. Agric. Food Chem.* **1971**, *19*, 130–133.

(8) Young, C. T. Influence of drying temperature at harvest on major volatiles released during roasting of peanuts. *J. Food Sci.* **1973**, *38*, 123–125.

(9) Lovegren, N. V.; Ninnett, C. H.; St. Angelo, A. J. Gas chromatographic profile of good quality raw peanuts. *Peanut Sci.* **1982**, *9*, 93–96.

(10) Young, C. T.; Hovis, A. R. A method for the rapid analysis of headspace volatiles of raw and roasted peanuts. *J. Food Sci.* **1990**, *55*, 279–280.

(11) Didzbalis, J.; Ritter, K. A.; Trail, A. C.; Plog, F. J. Identification of fruity/fermented odorants in high-temperature-cured roasted peanuts. *J. Agric. Food Chem.* **2004**, *52*, 4828–4833.

(12) Schirack, A. V.; Drake, M. A.; Sanders, T. H.; Sandeep, K. P. Characterization of aroma-active compounds in microwave blanched peanuts. *J. Food Sci.* **2006**, *71*, 513–520.

(13) Sanders, T. H.; Vercellotti, J. R.; Crippen, K. L.; Civille, G. V. Effect of maturity on roast color and descriptive flavor of peanuts. *J. Food Sci.* **1989**, *54*, 475–477.

(14) Williams, E. J.; Drexler, J. S. A non-destructive method for determining peanut pod maturity. *Peanut Sci.* **1981**, *8*, 134–141.

(15) Johnsen, P. B.; Civille, G. V.; Vercellotti, J. R.; Sanders, T. H.; Dus, C. A. Development of a lexicon for the description of peanut flavor. *J. Sensory Stud.* **1988**, *3*, 9–17.

(16) Meilgaard, M.; Civille, G. V.; Carr, B. T. Affective tests: consumer tests and in- house panel acceptance tests. In *Sensory Evaluation Techniques*, 3rd ed.; CRC Press: Boca Raton, FL, 1999; pp 231−236.

(17) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation−a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.

(18) Van Ruth, S. M.; O'Conner, C. H. Evaluation of three gas-chromatography−olfactometry methods: comparison of odour intensity−concentration relationships of eight volatile compounds with sensory headspace data. *Food Chem.* **2001**, *74*, 341–347.

(19) Grosch, W. Detection of potent odorants in foods using aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68–73.

(20) National Institute of Standards and Technology. NIST/EPA/NIH mass spectral library with search program, data version NIST02; Gaitherburg, MD, 2005.

(21) Van den Dool, H.; Kratz, P. A generalization of the retention index system including programmed gas liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463–471.

(22) McNeill, K. L.; Sanders, T. H. Maturity effects on sensory and storage quality of roasted Virginia-type peanuts. *J. Food Sci.* **1998**, *63*, 336–339.

(23) Uniform Peanut Performance Trials (UPPT). USDA-ARS-Market Quality and Handling Unit, 2006; http://152.1.118.33/.

(24) Matsui, T.; Guth, H.; Grosch, W. A comparative study of potent odorants in peanut, hazelnuts, and pumpkin seed oils on the basis of aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry of headspace samples (GCOH). *Fett/Lipid* **1998**, 51–56.

(25) Baker, G. L.; Cornell, J. A.; Gorbet, D. W.; O'keefe, S. F.; Sims, C. A.; Talcott, S. T. Determination of pyrazine and flavor variations in peanut genotypes during roasting. *J. Food Sci.* **2003**, *68*, 394–400.

(26) Avsar, Y. K.; Karagul-Yuceer, Y.; Drake, M. A.; Singe, T. K.; Yoon, Y.; Cadwallader, K. R. Characterization of nutty flavor in cheddar cheese. *J. Dairy Sci.* **2004**, *87*, 1999–2010.

(27) Whitaker, T. B.; Dickens, J. W. Effects of curing on respiration and off-flavor in peanuts. *Proceedings of the 3rd National Peanut Research Conference*, Auburn, AL, 1964.

(28) Whitaker, T. B.; Slate, A. B.; Greene, J. L.; Hendrix, K.; Sanders, T. H. Uncertainty associated with sampling peanuts to determine fruity-fermented off-flavor. *Peanut Sci.* **2006**, *34*, 126–134.

Received for review February 13, 2008. Revised manuscript received June 16, 2008. Accepted June 28, 2008.

JF800450K